The APOBEC3 Gene Family as Guardians of Genome Stability in Human Embryonic Stem Cells

Grant Award Details

The APOBEC3 Gene Family as Guardians of Genome Stability in Human Embryonic Stem Cells

Grant Type: SEED Grant

Grant Number: RS1-00210

Investigator:

Name:	Warner Greene
Institution:	Gladstone Institutes, J. David
Type:	PI

Disease Focus: Cancer

Human Stem Cell Use: Embryonic Stem Cell

Award Value: $717,363

Status: Closed

Progress Reports

Reporting Period: Year 2

View Report

Grant Application Details

Application Title: The APOBEC3 Gene Family as Guardians of Genome Stability in Human Embryonic Stem Cells

Public Abstract:

The successful use of human embryonic stem cells (hESCs) as novel regenerative therapies for a spectrum of currently incurable diseases critically depends upon the safety of such cell transfers. hESCs contain roughly 3 million “jumping genes” or mobile genetic retroelements that comprise up to 45% of their genetic material. While many of these retroelements have been permanently silenced during evolution by crippling mutations, many remain active and capable of moving to new chromosomal locations potentially producing disease-causing mutations or cancer. More mature differentiated cells control retroelement movement (retrotransposition) by methylating the DNA comprising these elements. Strikingly, such DNA methylation is largely absent in hESCs because these cells must be able to develop into a wide spectrum of different tissues and organs. Thus, in order to protect the integrity of their genomes, hESCs must deploy an additional defense to limit retroelement retrotransposition. Recent studies of HIV and other exogenous retroviruses have identified the APOBEC3 family of genes (A3A-A3H) as powerful anti-retroviral factors. These APOBEC3s interrupt the conversion of viral RNA into DNA (reverse transcription), a key step also used by retroelements for their successful retrotransposition. We hypothesize that one or more of the APOBECs function as guardians of genome integrity in hESCs. We propose to compare and contrast which APOBEC3s are expressed in one federally approved and nine nonapproved hESC lines and to assess the natural level of retroelement RNA expression occurring in each of these lines. Next we will test whether the knockdown of expression of these APOBEC3s in the hESCS lines by RNA interference leads to a higher frequency of retrolement retrotransposition. Finally, if higher levels of retrotransposition are detected, we will examine whether these cells display an impaired ability to differentiate into specific tissue types corresponding to the three germ cell layers (ectoderm, mesoderm, and endoderm) and whether increased retrotransposition is associated with a higher frequency of malignant transformation within the hESC cultures. These studies promise to provide important new insights into how genomic stability in is maintained in hESCs and could lead to the identification of specific GMP culture conditions that minimize the chances of such unwanted retrotransposition events in cells destined for infusion into patients. These studies are directly responsive to the CIRM request for application. If funded, these studies would allow the entry of my laboratory with extensive APOBEC experience, into the exciting field of stem cell biology.

Statement of Benefit to California:

Harnessing the exciting potential of embryonic stem cells as therapies for a wide range of diseases like diabetes, Alzheimer’s disease, myocardial infarction among others first requires ensuring that the infusion of these cells into patients can be performed safely. Of note, human embryonic stem cells contain up to 3 million “jumping genes” or mobile genetic retroelements that can potentially move from location to another in the genome. Great harm could occur if the movement of these retroelements in human embryonic stem cells results in the mutation of key genes or the inactivation of tumor suppressor genes, the latter could facilitate the development of cancer in recipients of these cells. The safety of stem cell therapy thus depends on the rigorous maintenance of genomic integrity and stability within the embryonic stem cell during its manipulation. Strikingly, the major cellular defense against the movement of the retroelements to new genetic locations, DNA methylation, is greatly reduced in human embryonic stem cells. A general state of hypomethylation is likely required to permit these pluripotent cells to differentiate into multiple cell types. With DNA methylation no longer able to constrain the activity of these retroelements, we believe a second natural defense springs into action to protect these stem cells. We proposeto identify and characterize this defensive network. These studies could lead to new approaches for maintaining or even enhancing this defense when embryotic stem cells are manipulated in culture, thereby helping to ensure the safety of embryonic stem cells destined for therapeutic transfer. Thus, the results of these studies will have both scientific and practical value. As such, we believe these studies will benefit the citizens of California certainly at a societal level and potentially at a personal level.